CLAIMS

1. A reference standard for a detectable entity, the reference standard comprising a support medium, preferably an embedding medium, a compact particle having a compact shape with a quantity of detectable entity coupled thereto and supported by the medium, in which the compact particle is a biological, preferably cellular compact particle, preferably a cellular compact particle.

2. A reference standard for a detectable entity, the reference standard comprising a support medium, preferably an embedding medium, a compact particle having a compact shape with a quantity of detectable entity coupled thereto and supported by the medium, in which the compact particle is a non-biological compact particle, preferably a non-cellular compact particle having cell-like dimensions, preferably less than 1.5 mm.

3. A reference standard according to Claim 1 or 2, in which a detectable amount of the detectable entity is present in a defined region in a cross section of the reference standard.

4. A reference standard according to Claim 1, 2 or 3, in which the detectable entity adopts a compact shape, preferably an unextended or non-elongate shape, in the support medium.

5. A reference standard according to any preceding claim, in which the compact shape is such that the ratio of the longest dimension to the shortest dimension is less than 5:1, preferably less than 2:1

6. A reference standard according any preceding claim, in which the compact shape comprises a particulate, uniform or regular shape.

7. A reference standard according to any preceding claim, in which the compact shape comprises a sphere shape, an ovoid shape, an ellipsoid shape, a disc shape, a cell shape, a pill shape or a capsule shape.

8. A reference standard according to any preceding claim, in which the detectable entity is heterologous to the compact particle.

9. A reference standard according to any preceding claim, in which the detectable entity is chemically coupled to the compact particle.

10. A reference standard according to any preceding claim, in which the compact particle comprises a cell.

11. A reference standard according to Claim 10, in which the cell does not express the detectable entity.

12. A reference standard according to Claim 10 or 11, in which the cell is selected from the group consisting of: a virus, a bacterial cell, a eukaryotic cell, an insect cell, an animal cell, a mammalian cell, a mouse cell and a human cell.

13. A reference standard according to Claim 10, 11 or 12, in which the cell comprises an insect cell, preferably an Sf9 cell, or a mammalian cell, preferably a Chinese Hamster Ovary (CHO) cell.

14. A reference standard according to any of Claims 1 to 9, in which the compact particle comprises an organelle.

15. A reference standard according to Claim 14, in which the organelle comprises a mitochondrion, a plastid, a chloroplast, or a nucleus, preferably derived from a cell as set out in any of Claims 11 to 13.

16. A reference standard according to any of Claims 1 to 9, in which the detectable entity is substantially free of cellular material.

17. A reference standard according to any of Claims 1 to 9 and 16, in which the compact particle comprises a microbead or a micelle.

18. A reference standard according to any preceding claim, in which the compact shape has a dimension of less than 1000μm, preferably less than 500μm, preferably less than 200μm, preferably less than 100μm, preferably less than 50μm, more preferably less than 20μm, most preferably less than 10μm.

19. A reference standard according to any preceding claim, in which the defined region is present in at least one other cross section of the reference standard, preferably comprising a similar amount of detectable entity.

20. A reference standard according to any preceding claim, in which the support medium comprises an embedding medium, in which the detectable entity is embedded.

21. A reference standard according to any preceding claim, in which the detectable entity comprises a diagnostically relevant target.

22. A reference standard according to any preceding claim, in which the detectable entity comprises an antigen, an epitope, a peptide, a polypeptide, a protein, a nucleic acid, or two or more or a plurality of any of the above, or combinations of one or more of the above.

23. A reference standard according to any preceding claim, in which the detectable entity is selected from the group consisting of: a hapten, a biologically active molecule, an antigen, an epitope, a protein, a polypeptide, a peptide, an antibody, a nucleic acid, a virus, a virus-like particle, a nucleotide, a ribonucleotide, a deoxyribonucleotide, a modified deoxyribonucleotide, a heteroduplex, a nanoparticle, a synthetic analogue of a nucleotide, a synthetic analogue of a ribonucleotide, a modified nucleotide, a modified ribonucleotide, an amino acid, an amino acid analogue, a modified amino acid, a modified amino acid analogue, a steroid, a proteoglycan, a lipid, a carbohydrate, a dye, and mixtures, fusions, combinations or conjugates of the above.

24. A reference standard according to any preceding claim, in which the detectable entity comprises any one or more of HER2, oestrogen receptor (ER), progesterone receptor (PR), p16, Ki-67, c-kit, laminin 5 gamma 2 chain and Epidermal Growth Factor Receptor (EGFR) protein, nucleic acids encoding such, and post-translationally modified forms, preferably phosphorylated forms of such.

25. A reference standard according to any preceding claim, in which the presence and/or quantity of the detectable entity is revealable by a binding agent, preferably a labelled binding agent.

26. A reference standard according to Claim 25, in which the binding agent is selected from the group consisting of: an antibody, preferably an antibody capable of specific binding to the detectable entity, a nucleic acid such as a DNA or an RNA, preferably a nucleic acid capable of specific binding to the detectable entity, a protein nucleic acid (PNA), a dye, a special stain, Au-chloride, Haematoxylin-Eosin (H & E), Gomori methenamine silver stain (GMS), Periodic Acid-Schiff (PAS) stain, Trichrome Blue, Masson's Trichrome, Prussian Blue, Giemsa, Diff-Quik, Reticulum, Congo Red, Alcian Blue, Steiner, AFB, PAP, Gram, Mucicarmine, Verhoeff-van Gieson, Elastic, Carbol Fuchsin and Golgi's stains.

27. A reference standard according to any preceding claim, in which the presence of the detectable entity in a cell, tissue, organ or organism is indicative of a disease or a condition.

28. A reference standard according to any preceding claim, in which the defined region includes a reference area, the reference area comprising the detectable entity at a pre-defined amount.

29. A reference standard according to Claim 28, in which the amount of the detectable entity in the reference area is compared to the amount of the detectable entity in a sample to determine the presence, quantity or concentration of the detectable entity in the sample.

30. A reference standard according to any preceding claim, in which the reference standard is in the shape of a rectangular box.

31. A reference standard for a detectable entity, comprising: (a) an embedding medium in a preferably substantially rectangular box shape; and (b) a cell with a quantity of detectable entity coupled thereto.

32. A reference standard according to any preceding claim, which comprises two or more compact particles, each having detectable entity coupled thereto.

33. A reference standard according to any preceding claim, which comprises two or more different detectable entities, each of which is coupled to the same or different compact particle.

34. A reference standard according to any preceding claim, which comprises two or more compact particles comprising different amounts of detectable entity on each.

35. A reference standard according to any preceding claim, in which a planar section of the reference standard comprises a plurality of areas on which are presented the detectable entity at different density.

36. A reference standard according to any preceding claim, in which a planar section of the reference standard comprises a first area comprising the detectable entity substantially at a diagnostically significant density.

37. A reference standard according to any preceding claim, further comprising a control comprising a compact particle which comprises substantially no detectable entity.

38. A reference standard according to any preceding claim, in which the embedding medium is selected from the group consisting of: ice, wax, paraffin, acrylic resin, methacrylate resin, epoxy, Epon, Araldite, Lowicryl, K4M and LR White and Durcupan.

39. A reference standard for a detectable entity comprising an surrounding medium together with a quantity of detectable entity located in the surrounding medium in a defined amount, in which the detectable entity adopts a compact shape in the surrounding medium.

40. A planar section, preferably a transverse planar section, preferably of substantially uniform thickness, of a reference standard according to any preceding claim.

41. A support, preferably a slide such as a microscope slide, comprising a planar section according to Claim 40 mounted thereon.

42. A kit comprising a reference standard according to any preceding claim, together with a binding agent capable of specific binding to the detectable entity, optionally together with instructions for use.

43. A reference standard, kit or a planar section according to any preceding claim, in which the reference standard has been stained, preferably with an antibody or a nucleic acid probe.

44. A diagnostic kit for detecting the presence or amount of a detectable entity in a biological sample, comprising: (a) a reference standard, planar section or slide according to any preceding claim; (b) a binding agent capable of specific binding to the detectable entity; and optionally (c) instructions for use.

45. A combination of a reference standard, planar section, support, kit or diagnostic kit according to any of Claims 1 to 44 together with a therapeutic agent capable of treating or alleviating at least one of the symptoms of a disease or condition in an individual.

46. A combination according to Claim 45, in which the individual is diagnosed as suffering from or susceptible to the disease or condition, if the amount of detectable entity in the biological sample or component is similar to or greater than that in the reference standard.

47. A diagnostic kit according to Claim 44 or a combination according to Claim 45 or 46, in which the binding agent or therapeutic agent comprises an antibody against the detectable entity.

48. Use of a reference standard, a planar section or a kit according to any preceding claim, for determining the presence or amount of a detectable entity in a biological sample.

49. A method of comparing the amount of a detectable entity in a biological sample with a reference standard, the method comprising the steps of:

(a) providing a biological sample and obtaining a first signal indicative of the amount of detectable entity in the biological sample, or a component thereof;

(b) providing a reference standard, planar section, support, kit or diagnostic kit according to any of Claims 1 to 44;

(c) obtaining a second reference signal indicative of the amount of detectable entity in the reference standard or planar section thereof; and

(d) comparing the first signal obtained in (a) against the reference signal.

50. A method according to Claim 49, in which the detectable signal is selected from the group consisting of: radiation, optical density, reflectance, radioactivity, fluorescence, enzymatic activity.

51. A method according to Claim 49 or 50, in which the reference standard or planar section thereof is subjected to the same one or more steps or conditions, preferably substantially all, as the biological sample.

52. A method according to Claim 49, 50 or 51, in which the reference standard or planar section thereof is processed through one or more, preferably all, of the following steps: mounting onto a slide, baking, deparaffination, rehydration, antigen retrieval, blocking, exposure to antibody, exposure to primary antibody, exposure to nucleic acid probe, washing, exposure to secondary antibody-enzyme conjugate, exposure to enzyme substrate, exposure to chromogen substrate, and counter staining.

53. A method or use according to Claims 48 to 52, in which the biological sample comprises a cell, tissue or organ, preferably a cell, tissue or organ of an organism suspected of suffering a disease or condition.

54. A method of diagnosis of a disease or a condition in an individual, the method comprising the steps of:

(a) obtaining a biological sample from the individual; and

(b) comparing the amount of a detectable entity in a biological sample or component thereof with a reference standard, in a method according to Claim 49;

in which preferably the individual is diagnosed as suffering from or susceptible to the disease or condition, if the amount of detectable entity in the biological sample or component is similar to or greater than that in the reference standard.

55. A method of treatment of a disease or a condition in an individual, the method comprising the steps of diagnosing the disease or condition in an individual in a method according to Claim 54, and administering a therapeutic agent to the individual.

56. A method of treatment according to Claim 55, in which the therapeutic agent comprises an antibody capable of binding to the detectable entity.

57. A method of assessing the effectiveness or success of a procedure, the method comprising the steps of:

(a) providing a reference standard according to any of Claims 1 to 39, in which a detectable property of the detectable entity is changed as a result of the procedure;

(b) conducting the procedure on the reference standard; and

(c) detecting a change in the detectable property of the detectable entity.

58. A method according to Claim 57, in which a detectable property of the detectable entity is changed as a result of a successful procedure, which change in the detectable property of the detectable entity is detected to establish that the procedure is successful.

59. A method according to Claim 57, in which a detectable property of the detectable entity is changed as a result of an unsuccessful procedure, which change in the detectable property of the detectable entity is detected to establish that the procedure is not successful.

60. A method of validating a procedure according to Claim 57, 58 or 59, in which the procedure is selected from the group consisting of: an *in situ* hybridisation procedure, an immunohistochemical procedure, deparaffination, antigen retrieval, blocking, endogenous biotin blocking, endogenous enzyme blocking, a washing step, incubation with revealing agent such as a primary antibody, incubation with secondary visualisation components, chromogen staining, staining information acquisition and analysis.

61. A method according to any of Claims 57 to 60, in which the procedure is an antigen retrieval procedure, and in which the detectable property of the detectable entity comprises the masking or unmasking of one or more epitopes.

62. A method according to any of Claims 57 to 61, in which the detectable entity in the reference standard is modified to mask one or more epitopes, some or all of which are unmasked in an antigen retrieval procedure which is successful.

63. A method according to any of Claims 57 to 60, in which the procedure is an deparaffination procedure, and in which the detectable property of the detectable entity comprises the presence or quantity of detectable entity in the reference standard following the deparaffination procedure.

64. A method according to any of Claims 57 to 60 and 63, in which the detectable entity in the reference standard is soluble in the deparaffination medium, and in which at least a portion, preferably all, of the detectable entity is removed following a successful deparaffination procedure.

65. Use of a reference standard as claimed in any preceding claim as an antigen retrieval validation standard, a deparaffination standard, a blocking validation standard, a washing validation standard, a primary antibody validation standard, a secondary antibody validation standard, a calibration standard, or a diagnostic standard.

66. A method of producing a reference standard for a detectable entity, the method comprising the steps of: (a) providing a support medium, preferably an embedding medium; (b) providing a compact particle having a compact shape, the compact particle having cell-like dimensions; (c) coupling a quantity of detectable entity to the compact particle and (d) supporting or embedding the compact particle in the medium.

67. A method of producing a reference standard for a detectable entity, the method comprising the steps of: providing a compact particle of biological, preferably cellular origin, and coupling a quantity of detectable entity to the compact particle.

68. A method of producing a reference standard for a detectable entity, the method comprising supporting a compact particle having a compact shape and cell-like dimensions having a quantity of detectable entity coupled thereto in a support medium.

69. A method of producing a reference standard for a detectable entity, the method comprising the steps of: (a) providing an embedding medium; (b) forming a quantity of detectable entity in a generally compact shape and cell-like dimensions by coupling to a compact particle, the compact particle having cell-like dimensions; and (c) embedding the detectable entity in the embedding medium.

70. A method according to any of Claims 66 to 69, which further comprises coupling a quantity of a second detectable entity to the compact particle, or to a second compact particle.

71. A method according to any of Claims 66 to 70, which further comprises coupling a second different quantity of the or each detectable entity to the or each compact particle.

72. A method according to any of Claims 66 to 71, in which the support medium comprises an embedding medium, and the or each compact particle is supported by embedding in the embedding medium.

73. A method according to any of Claims 66 to 72, in which the or each detectable entity is covalently coupled to its respective compact particle.

74. A reference standard for a detectable entity, comprising a detectable entity coupled to a cell and supported by a support medium.

75. A method of producing a reference standard for a detectable entity, the method comprising the steps of providing a cell, coupling a quantity of detectable entity to the cell, and embedding the cell in an embedding medium.

76. A reference standard according to Claim 74 or a method according to Claim 75, in which the cell does not express the detectable entity.

77. A method or reference standard according to Claim 74, 75 or 76, in which the cell is selected from the group consisting of: a virus, a bacterial cell, a eukaryotic cell, an insect cell, preferably an Sf9 cell, an animal cell, a mammalian cell, preferably a Chinese Hamster Ovary (CHO) cell, a mouse cell and a human cell.

78. An artificial cell or organelle comprising a detectable entity coupled to a compact particle having a compact shape.

79. A method of making an artificial cell or organelle comprising a detectable entity, the method comprising providing a compact particle having a compact shape, and coupling a quantity of detectable entity to the compact particle.

80. A modified cell or organelle comprising a detectable entity coupled to a cell, or a component thereof, which preferably does not express the detectable entity.

81. A method of making an modified cell or organelle comprising a detectable entity, the method comprising providing a cell or a component thereof which does not express the detectable entity, and coupling a quantity of detectable entity to the cell or component.

82. A method of establishing a cellular distribution of detectable entity in a reference standard, the method comprising providing a cell or a component thereof which does not express a detectable entity, coupling a quantity of detectable entity to the cell or component, and supporting the cell or component in a support medium.

83. A reference standard substantially as hereinbefore described with reference to and as shown in the accompanying drawings.

84. A planar section preferably of substantially uniform thickness of a reference standard substantially as hereinbefore described with reference to and as shown in the accompanying drawings.

85. Use of a reference standard or a planar section for determining the presence or amount of a detectable entity in a biological sample, such use substantially as hereinbefore described with reference to and as shown in the accompanying drawings.

86. A method of determining the amount of a detectable entity in a biological sample substantially as hereinbefore described with reference to and as shown in the accompanying drawings.

87. A method of diagnosis of a disease or a condition in an individual substantially as hereinbefore described with reference to and as shown in the accompanying drawings.